REMARKS

New Claims 40-50 correspond to canceled Claims 1-2, 6-12, 19-22, 24, and 35-36. The new claims have been amended to overcome the Examiner's 35 U.S.C. § 112 rejections.

1. The Invention

The present invention relates to novel recombinant adenoviruses characterized by at least two lethal deletions in early gene regions and the novel packaging cell lines that function to propagate these replication deficient adenoviruses. The deletion of two essential regions, both the E1 and E4 regions, dramatically minimizes or eliminates the pathogenic effects of direct cytotoxicity to the targeted cells and inflammatory responses in the human body. The resulting virus, however, is replication defective and requires the E1 and E4 functions in trans in order to replicate. However, since the expression of E1 activates the expression of E4 which is cytotoxic, no one has been able to develop a cell line that expresses and provides these functions to support viral replication and packaging.

The present invention provides a novel packaging cell line which complements functions of E1 and E4, and optionally the E3 DNA regions. The present invention overcomes the difficulty of establishing a cell line to complement the E1 and E4 functions deleted from the recombinant adenoviruses of the present invention by providing a 293 host cell which contains the E1a, E1b, E2a and E4 gene regions. The E4 gene region has been introduced into 293 cells and placed under the control of an inducible promoter, e.g., a CREB or a

tetracycline inducible promoter, so that in the uninduced state, expression is low enough to avoid toxicity to the host cell, but in the presence of tetracycline is sufficiently activated to make enough E4 gene product to complement the E4 deleted region during virus production.

2. The Rejections Under 35 U.S.C. §112 Should Be Withdrawn

The specification is objected to and Claims 9, 24, 38 and 39 are rejected under 35 U.S.C. §112 for lack of enablement. Applicants submit that these rejections are in error and should be withdrawn for the reasons explained below.

The Examiner asserts that the specification fails to enable the use of a tetracycline responsive promoter to drive the expression of an adenoviral protein. The Examiner's attention is invited to page 15 of the specification, lines 13 to 18 which describe the use of a tetracycline responsive promoter to regulate the expression of an adenoviral gene. The specification cites Manfred and Hermann (1992, Proc. Natl. Acad. Sci. 89:5547-5551) incorporated by reference in its entirety, which describes the construction and use of a tetracycline promoter. The reference contains a detailed description of the sequences which encode a tetracycline promoter, the construction of a tetracycline dependent promoter and the protocol to activate and inactivate the tetracycline promoter in order to tightly regulate expression of a target gene. Therefore, one of ordinary skill in the art would have ample guidance for the use of a tetracycline responsive promoter. Further, the studies described in the

Manfred and Hermann reference were carried out in HeLa cells. HeLa cells, as well as 293 cells, are competent for replication of adenovirus, therefore there is no reason to believe that this widely used promoter would not function effectively in 293 cells. Accordingly, the Applicants assert that the claims are fully enabled and request that the rejections under 35 U.S.C. §112, first paragraph, be withdrawn.

The Examiner asserts that the specification fails to provide an enabling disclosure for a cell line which can complement an E1/E2a/E4-deleted adenovirus. The Examiner specifically states "Because the regulation of these proteins (E1, E2a and E4) must be tightly controlled, it is not clear that one could create such a cell line." Applicants submit that this rejection is in error and should be withdrawn for the reasons explained below.

The Examiner's attention is invited to Example 1 of the instant specification (page 25, line 1 to page 26, line 10) which describes the construction of DNA plasmids to introduce the E4 gene region under the control of the regulatable promoter, the α-inhibin promoter, into 293 cells which express E1 and E2a; Example 2 (page 26, line 11 to page 27, line 1) which describes the transfection and selection process employed to establish 293-E4 cell lines. Example 3 (page 27, line 2 to line 16) and Example 9 (page 31, line 15 to page 32, line 26) which describe the detection of the E4 gene in 293 cells, in addition to the detection of E1, in order to demonstrate that the E1 gene was not altered in the 293-E4 cell lines; Example 10 (page 32, line 27 to page 33, line 21)

which describes the ability of the 293-E4 cell lines, which also express the E1 and E2a adenoviral proteins, to support the growth of E4-deleted adenoviruses; and Example 13 (page 36, line 20 to page 37, line 18) which describes the ability of the 293-E4 deleted adenoviruses. This disclosure describing the construction of cell lines which express E1, E2a and E4 and the ability of these cell lines to support replication of E1 and E4 deleted replication defective adenoviruses, demonstrates that the specification is clearly enabling to support the invention as claimed.

The Examiner further asserts that the specification fails to provide support for a packaging cell line that supports the growth of a replication defective recombinant adenovirus that carries a deletion of the adenovirus rep gene region. The claims have been amended to delete this feature, thereby obviating this rejection.

Therefore, the rejections under 35 U.S.C. §112, first paragraph are obviated and/or should be withdrawn.

3. The Rejections Under 35 U.S.C. §102 Should Be Withdrawn

Claims 11-12, 19-22 and 29-31 covering a novel replication defective adenovirus, novel recombinant adenoviruses defective in replication, novel packaging cell lines to support the growth of these viruses and a method of infecting mammalian target cells with these viruses containing a transgene, are rejected under 35 U.S.C. §102(a) as being anticipated by Engelhardt. The Examiner has also rejected

Claims 19-22 and 36-37 under 35 U.S.C. §102(a) as being anticipated by Armentano.

The claims have been amended to recite novel replication defective adenoviruses which contain at least two lethal deletions, two lethal mutations or one lethal deletion and one lethal mutation in the E1 and E4 early gene regions and cell lines to support such replication defective vectors. The vectors and cell lines disclosed in Englehardt and Armentano clearly are not encompassed by the claims as amended. Therefore, the rejections under 35 U.S.C. §102(a) have been obviated.

Claims 1-2 and 11-12 covering DNA plasmids comprising an adenoviral gene or gene region that encodes a cytotoxic protein under the control of an inducible promoter and packaging cell lines that support the growth of a replication defective adenovirus, are rejected under 35 §102(b) as being anticipated by Klessig. Applicants submit that these rejections have been obviated and should be withdrawn for the reasons explained below.

The legal test for anticipation under 35 U.S.C. §102 requires that each and every element of the claimed invention be disclosed in a prior art reference in a manner sufficient to enable one skilled in the art to reduce the invention to practice, thus placing the public in possession of the invention. W.L. Gore Associates v. Galock, Inc., 721 F.2d 1540, 1554 (Fed. Cir. 1983) cert. denied 469 U.S. 851 (1984); In re Donohue, 766 F.2d 531 (Fed. Cir. 1985). Anticipation under U.S.C. §102 requires identity of invention. Scripps

Clinic & Research Fdn. v. Genentech Inc., 927 F.2d 1565 (Fed. Cir. 1991).

In this instance, the invention described by the claims as amended cover DNA plasmids comprising an adenoviral E4 gene or E4 early gene region that encodes a cytotoxic protein operably linked to an inducible promoter; and novel replication-defective recombinant adenoviruses containing at least two lethal deletions in the early gene regions, in addition to novel packaging cell lines which supply the E1 functions as well as the cytotoxic E4 functions needed to support propagation of these replication-defective adenoviruses.

The cited reference, Klessig, describes a DNA plasmid comprising an adenoviral gene (E2a) encoding a cytotoxic protein (DBP) operably linked to an inducible promoter and an Ad5-infected HeLa cell line which could complement E1/E2a-deleted adenoviruses. The DNA plasmid encoding DBP and the Ad5-infected HeLa cell line clearly are not encompassed by the amended claims. Therefore, the Examiner's rejection should be withdrawn.

Claims 11-12 and 35 drawn to packaging cell lines that support the growth of recombinant adenoviral vectors defective in replication are rejected under 35 U.S.C. §102(b) as being anticipated by Graham et al. These claims have been amended to recite packaging cell lines that support the growth of recombinant adenoviral vectors that contain at least two lethal deletions, at least two lethal mutations or at least one lethal mutation and one lethal deletion selected from the group of E1, E2a and E4 early gene regions. The reference

cited by the Examiner, Graham et al., describes the characterization of 293 cells. As discussed in Applicants' last amendment, 293 cells will support the growth of adenoviruses containing deletions in the E1 and E4 regions, except for the essential region of E4-ORF6. Since ORF6 is the essential region of the E4 early gene region, a deletion in the E4 early gene region -- except for ORF6 -- does not constitute a lethal deletion or mutation. In addition, 293 cells could not support the growth of a replication defective adenovirus containing a lethal detection in the E4 early gene region, and thus are clearly not encompassed by the invention as claimed. Thus, the claims are not anticipated by the prior art and, therefore, the Examiner's rejections under 35 U.S.C. § 102 should be withdrawn.

4. The Rejections Under 35 U.S.C. 103 Should Be Withdrawn

All claims (Claims 1-2, 6-12, 19-22, 24, 29-32, and 35-37) covering a novel replication defective adenovirus, novel recombinant adenoviruses defective in replication, novel packaging cell lines to support the growth of these viruses and a method of infecting mammalian target cells with these viruses containing a transgene, are rejected under 35 U.S.C. §103 as obvious over Weinberg, Gregory, Su and Pei.

Briefly, the Examiner contends that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the plasmid described by Weinberg, containing the promoter of Su and Pei, to stably transfect 293 cells thereby allowing for the production of E1/E4-deleted

adenoviruses of the present invention. The Examiner also contends that Gregory teaches that a cell line which complements both an E1 and E4 deletion in an adenoviruses could be established.

A finding of obviousness under § 103 requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the claimed subject matter and the prior art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere 383 U.S. 1 (1966). The relevant inquiry is whether the prior art suggests the invention, and whether the prior art provides one of ordinary skill in the art with a reasonable expectation of success. In re O'Farrell 853 F.2d 894, 903 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art and not in the Applicants' disclosure. In re Vaeck 947 F.2d 488 (Fed. Cir. 1991).

In the present instance, the relevant inquiry is, first, whether the cited art suggests (1) that the deletion of both E1 and E4 is required to completely block replication of adenovirus, and (2) how to engineer a "non-suicidal" packaging cell line which supplies both the E1 and E4 functions. Moreover, assuming arguendo that the prior art provided such a suggestion, the second inquiry is whether it provides one of ordinary skill in the art with a reasonable expectation of success. In re O'Farrell; In re Vaeck, supra. Applicants assert, however, that the prior art neither suggests the adenoviruses of the present invention containing two lethal

deletions and the cell lines of present invention which provide these functions, nor gives any reasonable expectation of success.

The Examiner's rejection relies on his contention that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the plasmid described by Weinberg to stably transfect 293 cells thereby allowing for the production of E1/E4-deleted adenoviruses. The Examiner's rejection further relies on the contention that Gregory teaches that a cell line could be established which complements both an E1 and E4 deletion in an adenoviral vector and provides an expectation of success. The Examiner's contention is in error.

The Examiner's contention is based on several erroneous assumptions. First, the art simply does not provide the suggestion of deleting both E1 and E4. Not one of the references relied on by the Examiner recognizes the importance of deleting both E1 and E4 in order to completely block adenovirus replication. The references recognize an advantage of deleting non-essential open reading frames of adenovirus in order to create more room to insert transgenes, but not the importance of deleting essential regions to completely block virus replication and the pathogenic effects. In fact, Weinberg's choice of Vero cells which do not complement E1 mutations pointedly excludes the use of E1/E4-deleted vectors.

Further, Gregory does not teach methods for producing adenoviruses with two lethal deletions. The adenoviruses disclosed in Gregory contain deletions in E1 or E3 in combination with deletions of the non-essential open reading

frames of E4. Gregory suggests deleting non-essential regions of E4 in order to enhance cloning capacity, but retains the essential region of E4 in order to maintain E4 functions in the virus. The deletions of non-essential regions do not constitute the lethal deletions of E1 and E4 in the replication defective adenoviral vectors of the present invention. Gregory does not teach the use of adenoviruses with deletions of the essential regions of E1 and E4, nor does he provide an expectation of success, because it was not known how to provide both of these functions in a "non-suicidal" packaging cell line.

The passage that the Examiner specifically refers to in Gregory on page 51 is basically a wish list on the part of Gregory. Gregory would like to be able to delete more nucleotide sequences in the E4 region in order to further enhance cloning capacity, but can not, because it was not known how to supply both E1 and E4 functions in a "non-suicidal" packaging cell line. Gregory recognizes the problem with establishing a cell line that would provide both of these functions, i.e., the E4 promoter is activated by the E1a gene product and E4 is toxic to the cell. Gregory speculates the transcription of E4 might be controlled by a transactivating system, but he provides no suggestion or guidance of how to accomplish this goal. Therefore, Gregory and Weinberg, either alone or in combination, do not provide the suggestion of creating an adenovirus containing two lethal deletions, nor do they solve the problem of establishing a packaging cell line which provides the deleted functions.

As discussed in Applicants' last amendment, there is no motivation to combine Su and Pei with Gregory and Weinberg. Su and Pei describe the characterization of the gene sequence of the inducible promoter of the murine inhibin α gene, but there is no suggestion that this promoter could be used to regulate the transcription of the cytotoxic protein E4 in 293 cells. The Examiner states that one would have been motivated to use the promoter of Su and Pei since it was well known at the time that promoters containing cAMP responsive elements inducibly regulated gene expression. However there were many inducible promoters known at the time. Therefore the suggestion lacking in Weinberg and Gregory is certainly not provided by Su and Pei.

Even assuming arguendo that the cited art suggests the invention, one of ordinary skill in the art would <u>not</u> have a reasonable expectation of success. As the Examiner states in the Office Action: "Because the regulation of these proteins (E1, E2a and E4) must be tightly controlled, it is not clear that one could create such a cell line". (Examiner's Office Action page 3, lines 17-19). In response to the Examiner's assertion that Gregory provides an expectation of success, even the Examiner who has combined the prior art to come up with the suggestion of creating the cell lines of the invention, still does not believe that it would be possible, due to the tight control of gene expression required. Therefore, even assuming arguendo the cited art provides a suggestion of the invention, <u>the cited art provides no expectation of successfully engineering the packaging cell lines of the present invention</u>.

In view of the foregoing, the art relied on by the Examiner does not render obvious the replication-defective adenoviruses and packaging cell lines of the claimed invention.

CONCLUSION

Applicants respectfully request entry and consideration of the foregoing amendments and remarks. The claims are believed to be free of the art, and patentable. Withdrawal of all the rejections and objections and an early allowance is earnestly sought.

Respectfully submitted,

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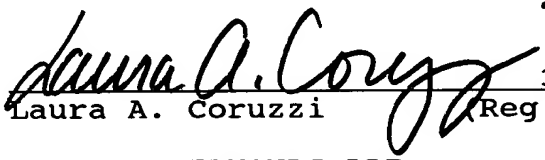

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Enclosure